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Trophic upgrading via the microbial food web may link terrestrial dissolved organic matter to *Daphnia*

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Running head: Trophic upgrading of DOM for *Daphnia*

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Abstract

Direct consumption of allochthonous resources generally yields poor growth and reproduction in zooplankton, but it is still unclear how trophic upgrading of terrestrial dissolved organic matter (tDOM) via microbial food web may support zooplankton. We compared survival, somatic growth, and reproduction of *Daphnia magna* fed with heterotrophic flagellate *Paraphysomonas vestita* and three algal diets. *Paraphysomonas* was fed lake bacteria that used tDOM as a substrate to simulate an allochthonous diet that zooplankton encounter in lakes. The highest survival, growth, and reproduction was achieved with a diet of *Cryptomonas*, while *Daphnia* performance was the worst when fed *Microcystis*. *Paraphysomonas* and *Scenedesmus* diets lead to intermediate growth and reproduction. *Cryptomonas* contained high amounts of essential polyunsaturated fatty acids (PUFA) and phytosterols that supported high somatic growth and reproduction, whereas poor performance of *Daphnia* on cyanobacterial diet was most likely due to lack of sterols. *Paraphysomonas* contained some phytosterols, but not in sufficient amounts, and also essential PUFA (eicosapentaenoic and arachidonic acid) that enhance zooplankton growth and reproduction. Our results indicate that tDOM-based microbial food web supports *Daphnia* performance even as a sole food source, and may be important in providing zooplankton with essential biochemical components when phytoplankton quantity or quality is low.

Introduction

The recognition that consumers in aquatic ecosystems may be fueled by terrestrial food sources in addition to in-lake phytoplankton production has sparked vast amount of research during the past three decades. The extent and possible pathways of consumer allochthony have been studied both under laboratory conditions and in the field (e.g. Grey et al. 2001; Pace et al., 2004; Berggren et al., 2014; Taipale et al., 2014). Field studies utilizing stable isotope ratios (C, N, H) have found that large fraction of consumer biomass can be traced to allochthonous sources (Pace et al., 2004; Berggren et al., 2014; Tanentzap et al. 2017, and references therein). Zooplankton allochthony may also vary seasonally following the relative availability of phytoplankton and allochthonous food sources (Grey et al. 2001). However, laboratory feeding experiments have questioned the feasibility of high zooplankton allochthony. Although zooplankton (mainly *Daphnia*) can survive on purely allochthonous diets, their growth efficiency, somatic growth rate and reproductive output is very low on allochthonous compared to phytoplankton diets (Brett et al., 2009; Wenzel et al., 2012; Taipale et al., 2014). Consequently, high inputs of terrestrial carbon and high consumer allochthony have been linked to low production of wild zooplankton (Kelly et al., 2014) and fish (Rask et al., 2014; Karlsson et al., 2015).

Most laboratory feeding trials testing consumer allochthony have been conducted using terrestrial particulate organic matter (tPOM) as the food source. More than 90% of terrestrial organic matter in lakes is in the dissolved form (DOM) (Kortelainen et al., 1993; Mattsson et al., 2005), and tPOM entering the lake in the shoreline or via river flow may rapidly sediment out of the water column. Thus, pelagic consumers especially in large lakes may have limited access to tPOM. Terrestrial DOM (tDOM) can be used as a substrate by bacteria, which can be grazed by heterotrophic protists including flagellates and ciliates (the microbial loop) or directly by zooplankton (Tranvik, 1992; Weisse 2004). *Daphnia* have been shown to benefit from tDOM directly or via tDOM-supported bacteria when algae is limiting (McMeans et al., 2015). Previous studies (Wenzel et al., 2012; Taipale et al., 2014) have found that *Daphnia* performance is better when feeding on mixtures of phytoplankton and bacteria than on mixtures of phytoplankton and tPOM, suggesting that DOM may be the more probable pathway for allochthonous organic matter to enter the grazer food web. According to feeding experiments, bacteria alone cannot support *Daphnia* growth and some taxa may even be toxic to *Daphnia* as a sole food source (Taipale et al., 2012; Freese and Martin-Creuzburg 2012). Few studies have been conducted on *Daphnia* performance on diets of heterotrophic flagellates, but results have been variable (Sanders et al., 1996; Bec et al., 2003; 2006).

One of the reasons proposed why *Daphnia* has poor growth on allochthonous diets is their lack of essential biomolecules, especially polyunsaturated fatty acids (PUFA) and sterols (Brett et

al., 2009; Taipale et al., 2014). Compared to many algae, tPOM contains very little PUFA, while bacteria contain none (Lechevalier and Lechevalier 1988; Taipale et al., 2014). Some studies have found that the fatty acid composition of heterotrophic flagellates depends on whether they feed on algae or bacteria (Zhukova and Kharlamenko, 1999; Véra et al., 2001) while others conclude that biosynthesis of lipids produces a consistent fatty acid (and sterol) composition in flagellates irrespective of diet (Bec et al., 2010; Parrish et al., 2012). Bacteria, including cyanobacteria, also lack sterols while phytoplankton contain various sterols in composition that is species-specific (Taipale et al., 2016). The sterol composition of flagellates is poorly studied, but so far studies have indicated that heterotrophic flagellates are capable of sterol synthesis (Klein Breteler et al., 1999; Bec et al., 2006). In addition to concentrating PUFA and sterols present in their food e.g. by selective retention, heterotrophic flagellates may enhance low quality bacterial or cyanobacterial food for *Daphnia* by either biosynthesizing PUFA and sterols *de novo* or modifying dietary short-chain PUFA to eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3). This so called 'trophic upgrading' by heterotrophic flagellates has been observed in several studies (Klein Breteler et al., 1999; Veloza et al., 2006; Bec et al., 2006; 2010). Also, indirect evidence of trophic upgrading was obtained when increased abundance of *Paraphysomonas vestita* in a decaying *Microcystis* culture was associated with rising EPA and DHA concentrations with a concurrent decrease in short-chain PUFA prominent in *Microcystis* (Park et al., 2003).

Previous studies on *Daphnia* performance on allochthonous diets have used tPOM, single strains of bacteria grown in artificial growth media, or heterotrophic flagellates growing on these bacteria as a diet source (but see McMeans et al., 2015). We conducted a feeding experiment where we constructed a simple microbial food web of tDOM (peat extract)-natural lake bacteria-*Paraphysomonas vestita* to better simulate the pathway for allochthonous carbon to enter zooplankton diets in lakes. We compared *Daphnia* somatic growth and reproduction on this allochthonous diet to diets of three phytoplankton taxa (*Cryptomonas*, *Scenedesmus*, *Microcystis*) known to vary in their quality as food for *Daphnia*. Our hypothesis was that 1) *Daphnia* survival would be better on tDOM-based microbial diet than on pure bacterial diets (as seen in other studies) and 2) *Daphnia* somatic growth and reproduction would be lower than on the algal diets.

Method

Experimental set up

We compared survival, growth and reproduction of *Daphnia* feeding on either diets of algae or a diet of a heterotrophic flagellate that was grown on bacteria utilizing tDOM as a substrate. For the experiment, we used *Daphnia magna* clone (DK-35-9), that originated from a pond in North Germany and has been raised successfully in laboratory for several years. Prior to the

experiment *Daphnia* were reared several generations on *Scenedesmus*. *Daphnia* neonates (<24h old) of multiple moms were pooled and randomly distributed among treatments (20 ind./treatment) and some were used to determine *Daphnia* mean initial body weight. During the feeding experiment, individual *Daphnia* were raised in 40mL vials in ADaM medium (Klüttgen et al., 1994). *Daphnia* were maintained on one of five different diets: no food, *Cryptomonas marssonii*, *Scenedesmus gracilis*, *Microcystis* sp. (strain 130, unicellular, non-toxic) or the heterotrophic flagellate *Paraphysomonas vestita*. The three algae were cultured in growth media optimal for each of them (Table 1) in 14h:10h light:dark cycle at 20ºC. The heterotrophic flagellate was grown in a culture medium containing tDOM extracted from unfertilized garden peat (Kekkilä luonnonturve) which was inoculated with lake bacteria (1 mL of 0.2 µm filtered lake water) a few days prior to addition of the flagellate. *Paraphysomonas* was concentrated with gentle centrifugation, but the diet given to *Daphnia* likely contained also bacteria.

The media was changed and the *Daphnia* fed every other day. We offered the food at non-limiting concentration: 1.5 mg C L^{-1} on days 0-2, 2 mg C L^{-1} on day 4 and 5 mg C L^{-1} from day 6 onwards. Every day *Daphnia* were inspected and dead animals, and the number of offspring were recorded. Sampling was conducted in the middle and in the end of the experiment (days 7 and 14), and *Daphnia* (10 ind.) in each treatment were collected for measurements of length, weight and subjected to fatty acid analysis. Due to difficulties in culturing the heterotrophic flagellate, we ended the *Paraphysomonas* treatment already after 12 days. To facilitate the comparison with *Daphnia* in algal diets that lasted 14 days, the eggs and embryos in *Daphnia* brood pouch in the *Paraphysomonas* treatment on day 12 were included as "potential neonates" for day 14.

Fatty acid and sterol analysis

Prior to analysis, *Daphnia* and food sources were freeze-dried and samples of 0.5-2.0 mg were weighed and placed in clean centrifuge tubes. Lipids were extracted with 2:1 chloroform:methanol following Folch et al. (1957) and the homogenization of tissues was facilitated with ultrasonic bath. After addition of 0.88 % aqueous KCl (w/v), the sample was thoroughly mixed and then centrifuged to separate the phases, and the chloroform phase containing the lipids was recovered. More chloroform was added, and the sample was extracted for the second time. The chloroform phases were pooled, and evaporated into dryness under N_2. At this point, the diet samples were split into two for consequent analysis of fatty acids and sterols.

Fatty acid methyl esters (FAMEs) were produced with acid catalyzed transesterification using methanolic H_2SO_4 (1%, v/v). The samples were placed in a heat block at 95ºC for 90 min. After they had cooled down, aqueous $KHCO_3$ (2%, w/v) was added to stop the reaction and the samples were extracted with 1:1 diethyl ether:n-hexane (v/v) to recover the FAMEs.

FAMEs were dissolved in n-hexane and analyzed with a gas chromatograph equipped with a mass-selective detector (GC-MS, Agilent). The column was DB-WAX (Agilent, length 30 m, ID 0.25 mm, film 0.25 µm) and helium was used as a carrier gas with a flow of 50.1 ml min^{-1}. Sample was injected splitless at 250ºC. The oven temperature program was as follows: 60 °C for 0.5 min, raised 15 °C min^{-1} to 180 °C and then 3°C min^{-1} to 210 where it was held for 30 min. FAMEs were identified based on their retention times and mass spectra. The response of the MS was corrected using known concentrations of a standard FAME mix (GLC 68E, Nu-Chek Prep, Inc.) and quantification was done in relation to an internal standard (21:0, 5 µg/sample).

Sterols were analyzed according to Taipale et al., (2016). Briefly, the extracted lipids were saponified with KOH at 70 °C, after which they were silylated with *N,O-bis*[trimethylsilyltrifluoro-acetamide] (BSTFA), trimethylchlorosilane (TMCS) and pyridine at 70°C. Trimethylsilyl (TMS) derivatives of sterols were analyzed with GC-MS (Shimadzu) equipped with A Phenomenex ZB-5 Guardian column (30 m × 0.25 mm × 0.25 µm). Sterols were identified using characteristic ions (Taipale et al., 2016).

Sterol index calculation

For defining if *Daphnia* somatic growth was suppressed by the lack of sterols, we calculated a sterol index based on the threshold values in Martin-Creuzburg et al., (2014) where growth-limiting thresholds for 10 different phytoplankton-derived sterols were determined. For sterols not included in the study of Martin-Creuzburg et al., (2014) we used the thresholds of the structurally most similar sterol (based on double bonds and carbon chain length). Threshold was 3.9 µg STE mg C^{-1} for fucosterol, 5.48 µg STE mg C^{-1} for brassicasterol, 8.25 µg STE mg C^{-1} for stigmasterol, 15.0 µg STE mg C^{-1} for camptesterol, and 21.7 µg STE mg C^{-1} for fungisterol, chondrillasterols, dihydrochondrillasterol and schottenol, and 22.0 µg STE mg C^{-1} for b-sitosterol. The sterol indexes were calculated using the following equation:

$$STE\ index = \frac{STE\ A}{REQ\ STE\ A} + \frac{STE\ B}{REQ\ STE\ B} + \cdots + \frac{STE\ Z}{REQ\ STE\ Z}$$

where STE A is the content of sterol A in food source and REQ STE A is the required amount of sterol A for optimal somatic growth. STE index values of >1 indicate that *Daphnia* growth is not limited by sterol availability.

Statistical analysis

Somatic growth rate (g) of *Daphnia* was calculated using the equation $g = (\ln W_1 - \ln W_0)/t$, where W_0 is the mean dry weight at the beginning of the experiment and W_1 after 14 days, and t is time (in days). Differences in growth rate and in final length of *Daphnia* among diet

192 treatments were tested with one-way analysis of variance (ANOVA). Length was log(k-L)
193 transformed (k = maximum value of length in the data +1, and L = length) to ensure the normal
194 distribution of residuals.
195 Fatty acid composition of *Daphnia* within a diet treatment was very similar in day 7 and
196 day 14 samples and the samples were pooled for further analysis. Differences in *Daphnia* total fatty
197 acid concentration between diet treatments was tested with non-parametric Kruskal-Wallis test.
198 Differences and similarities in *Daphnia* and diet fatty acid composition were illustrated with non-
199 metric multidimensional scaling (NMS) and tested with permutational multivariate analysis of
200 variance (PERMANOVA). PERMANOVA and NMS was run with euclidean distances.
201 PERMANOVA used type III sum of squares, unrestricted permutation of raw data, and the diet
202 treatment was handled as a fixed factor.
203
204 **Results**
205 *Survival and somatic growth*
206 All *Daphnia* died in seven days in the no food treatment (Fig. 1), and were not included in
207 further comparisons of growth, reproduction, and fatty acid composition. By comparison, all
208 *Daphnia* that were fed either *Scenedesmus* or *Cryptomonas* were alive at the end of the experiment
209 (day 14). 50% of *Daphnia* were alive in *Microcystis* diet treatment and 90% in *Paraphysomonas*
210 treatment at the end of the experiment (days 14 and 12, respectively).
211 Somatic growth rate of *Daphnia* was highest when fed *Cryptomonas* and *Scenedesmus*,
212 and lowest when fed *Microcystis* (ANOVA, $F_{3,16} = 85.18$, $p < 0.001$, Fig. 2A). The growth rate did
213 not differ statistically between *Paraphysomonas* and *Scenedesmus* treatments. Total length of
214 *Daphnia* differed among all diet treatments, and was highest in *Cryptomonas* (3.7 ± 0.1 mm)
215 treatment and lowest when fed *Microcystis* (2.1 ± 0.2 mm) (ANOVA, $F_{3,26} = 46.35$, $p < 0.001$, Fig.
216 2B).
217
218 *Reproduction*
219 Reproductive success varied greatly in *Daphnia* fed different diets. 100% of *Cryptomonas*-
220 fed and 90% of *Scenedesmus*-fed *Daphnia* produced neonates compared to only 10% when fed
221 *Microcystis*. In the *Paraphysomonas* treatment, 20% of *Daphnia* had produced neonates by day 12
222 but when also considering the eggs/embryos in the brood pouch the percentage increases to 80%.
223 The cumulative number of offspring produced during the experiment varied greatly among
224 individuals and diet treatments (Fig. 3). When feeding on *Cryptomonas Daphnia* produced 37.2 ±
225 14.2 (mean ± SD) neonates in 14 days, while feeding on *Scenedesmus* 16.4 ± 14.5 neonates, and on
226 *Microcystis* 0.6 ± 1.3 neonates. *Paraphysomonas*-fed *Daphnia* had produced 2.8 ± 5.6 neonates by

day 12, and may have produced up to 15.7 ± 12.0 by day 14 based on the number of eggs/embryos in the brood pouch.

Fatty acid and sterol composition of Daphnia and diets

Daphnia fatty acid composition resembled that of the food sources except for the *Microcystis* treatment (Fig. 4, Supplemental table S1) and differed statistically among the diet treatments (PERMANOVA, $F_{3,12} = 143.63$, $p < 0.001$). *Scenedesmus* and *Daphnia* feeding on *Scenedesmus* were characterized by high proportions of C18 PUFA, C16 PUFA and no detectable EPA. *Cryptomonas* contained high proportions of EPA, which was also seen in *Daphnia* feeding on *Cryptomonas*. *Paraphysomonas*, and *Daphnia* fed *Paraphysomonas*, were characterized by high proportions of 16:1ω7 and 18:1ω7 as well as moderate proportions of arachidonic acid (ARA, 20:4ω6) and EPA. *Microcystis* contained high proportions of 16:0 and 18:3ω6, but both of these fatty acids were markedly lower in *Daphnia* feeding on *Microcystis*. Instead, *Microcystis*-fed *Daphnia* exhibited high proportions of C16 and C18 monounsaturated fatty acids (MUFA) and ARA.

Total fatty acid concentration was much lower in *Daphnia* feeding on *Microcystis* (10.0 and 24.2 µg mg DW^{-1} for the two replicate analysis) or *Paraphysomonas* (27.1 ± 7.6 µg mg DW^{-1}) compared to 46.6 ± 5.6 µg mg DW^{-1} in *Scenedesmus* treatment (Kruskal-Wallis, $H = 11.61$, $n = 16$, $p = 0.009$, and pairwise comparisons). Total fatty acid concentration with *Cryptomonas* diet (38.4 ± 6.3 µg mg DW^{-1}) did not differ compared to the other treatments ($p > 0.05$).

Cryptomonas contained the highest concentration of sterols among the diets and contained mostly stigmasterol and brassicasterol (41.8% and 37.2%, respectively) (Table 2). *Paraphysomonas* contained sterols in similar amount as *Scenedesmus*, but they differed in composition. *Paraphysomonas* comprised mostly of β-sitosterol (92.8%) whereas *Scenedesmus* contained mainly chondrillasterol and fungisterol (58.6% and 23.5%, respectively). *Microcystis* did not contain any sterols. Sterol index was above 1.0 only for *Cryptomonas* (1.21 ± 0.05), and was low for *Scenedesmus* (0.21 ± 0.01) and *Paraphysomonas* (0.23 ± 0.01).

Discussion

Our study revealed that survival, growth and reproduction of *Daphnia* was markedly better when feeding on the heterotrophic flagellate *Paraphysomonas* supported by tDOM-based microbial food web than on the bloom-forming cyanobacteria *Microcystis*. *Daphnia* were bigger when fed *Paraphysomonas* although they were two days younger at the sampling time than when fed *Microcystis*. Overall, the best performance of *Daphnia* was achieved when fed the eukaryotic algae *Cryptomonas*, indicating that *Cryptomonas* has a sufficient content of essential fatty acids and

phytosterols for *Daphnia*, in line with previous studies (Martin-Creuzburg et al., 2008; Brett et al., 2009; Taipale et al., 2014).

Comparing the reproductive output of *Daphnia* in different diet treatments was challenging because, due to difficulties in culturing *Paraphysomonas*, we had to end the treatment two days earlier than the algal treatments. Two days is a long time in *Daphnia* reproductive cycle, and thus to facilitate the comparison of reproductive performance among treatments we included the embryos and eggs in the brood pouch of *Paraphysomonas*-fed *Daphnia* as "potential neonates" for day 14. The inclusion of eggs and embryos reveals that mean number of offspring produced in 14 days by *Daphnia* feeding on *Paraphysomonas* may be close to that when feeding on *Scenedesmus*, although markedly lower than when feeding on *Cryptomonas*. Our results suggest that microbial food web support *Daphnia* growth and reproduction reasonably well, even when offered as a sole food source.

Previous studies have shown that *Daphnia* survival, growth and reproduction are highly dependent on the biochemical composition of their food, more specifically on the PUFA and sterol content (Martin-Creuzburg and von Elert 2004; Taipale et al., 2014). In our experiment, the heterotrophic flagellate *Paraphysomonas* contained both sterols and essential PUFA, although in lower quantities than the eukaryotic algae. Fatty acid profile of *Paraphysomonas* was characterized by 16:1ω7 and 18:1ω7, both of which are common in bacteria and presumably originate in the diet of the flagellates, and the levels of these fatty acids were also elevated in *Daphnia*. Previous studies have shown that *Paraphysomonas* is capable of *de novo* synthesis of PUFA and sterols if they are not present in the diet (Bec et al., 2006; 2010). Although in general *Paraphysomonas* was poor in PUFA (~5% of all fatty acids) compared to the algal diets (~30-45%) *Paraphysomonas* provided *Daphnia* with EPA and ARA, which are important precursors of eicosanoid synthesis (Schlozt et al., 2012). Eicosanoids are hormone-like compounds that have a vital role e.g. in *Daphnia* reproduction (Schlozt et al., 2012).

Microcystis diet led to high mortality, and failed to support *Daphnia* growth and reproduction although it contained the C18 PUFA 18:3ω6, 18:3ω3, and 18:3ω4, the latter two being also key components in *Scenedesmus* that clearly was better quality food for *Daphnia* than *Microcystis*. What are lacking in *Microcystis* are sterols. Sterols are non-substitutable resources for *Daphnia*, and sterol limitation leads to impaired growth and reproduction (Martin-Creuzburg and von Elert, 2004; Martin-Creuzburg et al., 2008). In contrast to *Microcystis*, *Cryptomonas*, *Scenedesmus*, and *Paraphysomonas* all contained phytosterols, although their composition of sterols differed. *Cryptomonas* contained high quality phytosterols (brassicasterol and stigmasterol) that supported *Daphnia* somatic growth and reproduction efficiently. Both *Scenedesmus* and *Paraphysomonas* contained mainly sterols of lower quality and thus higher amount of these sterols

297 is required for optimal somatic growth and reproduction than the sterols in *Cryptomonas*. This is
298 reflected in *Cryptomonas* having a sterol index of 1.21 ± 0.05 and *Scenedesmus* and
299 *Paraphysomonas* 0.21 ± 0.01 and 0.23 ± 0.01, respectively. Previous studies on *Paraphysomonas*
300 have presented a somewhat different sterol profile with abundant poriferasterol/stigmasterol and
301 isofucosterol (Bec et al., 2006) compared to our results of β-sitosterol being the most abundant
302 sterol in *Paraphysomonas*. The sterol profile reported by Bec et al., (2006) would result in
303 *Paraphysomonas* having a much higher sterol index (1.73 ± 0.30) than any of the food sources in
304 our study. Nevertheless, Klein Breteler (1999) also found sterols (especially cholesterol and
305 brassicasterol) to be abundant in the marine heterotrophic dinoflagellate *Oxyrrhis marina*. So far, it
306 seems that heterotrophic flagellates can be a relatively good source of sterols for metazoan
307 consumers but more studies on different taxa are still needed to confirm their nutritional quality in
308 general. The biomass of heterotrophic flagellates generally is 10-fold lower than biomass of
309 phytoplankton in lakes (Auer et al., 2004), indicating that more sterols are available for zooplankton
310 from sterol-rich phytoplankton (e.g. *Cryptomonas*) than from heterotrophic flagellates. However,
311 heterotrophic flagellates may be an important food source seasonally, when phytoplankton biomass
312 e.g. during autumn and winter is low.
313 *Paraphysomonas* in our experiment was feeding on a bacterial community using tDOM
314 (peat extract) as a substrate. In general, bacteria do not contain PUFA or sterols (Lechevalier and
315 Lechevalier 1988; Volkman 2002) and previous studies show that pure bacterial diets are
316 detrimental for *Daphnia* (Taipale et al., 2012; Freese and Martin-Creuzburg 2012). Our results
317 indicate that *Paraphysomonas* is upgrading the bacterial food for *Daphnia* by synthesizing these
318 essential biochemicals *de novo* leading to higher survival, growth and reproduction of *Daphnia*. Our
319 results thus corroborate previous findings where *Paraphysomonas* has been shown to upgrade
320 cyanobacterial food for *Daphnia* (Bec et al., 2006; 2010; but see Bec et al., 2003). In an experiment
321 by McMeans et al. (2015) *Daphnia* growth rate and reproduction was very low when fed low
322 quantity of algae supplemented with tDOM and tDOM-supported bacteria (but without protists).
323 This further highlights the need of an additional trophic step when relying on terrestrial food
324 resources.
325 Pure diets are unrealistic in nature, but they do provide a useful indicator of quality of
326 different food items without other confounding factors. In accordance with previous findings
327 (Martin-Creuzburg et al., 2008), our results suggest that during cyanobacteria blooms, *Daphnia*
328 growth and reproduction may be severely compromised if alternative food sources do not exist.
329 Furthermore, cycling of organic matter through the microbial loop during blooms (Kluijver et al.,
330 2012) may markedly improve *Daphnia* performance due to trophic upgrading of biochemically
331 inadequate cyanobacterial food by *Paraphysomonas* (and potentially other heterotrophic

flagellates). *Paraphysomonas* may directly feed on small-celled cyanobacteria, or on the heterotrophic bacteria that use DOM leaching from cyanobacteria as a substrate. *Paraphysomonas* was shown to upgrade decaying *Microcystis* for *Daphnia* in a laboratory experiment (Park et al., 2003).

Terrestrial POM originating in leaves of riparian plants has been shown to be a very poor resource for *Daphnia*, due to low PUFA and sterol content and high content of non-digestible lignin (Brett et al., 2009; Taipale et al., 2014; 2016). Similarly, Wenzel et al., (2012) found that particulate peat as sole food source could not support *Daphnia* survival, growth or reproduction. Our study suggest that tDOM (leached from peat) entering the microbial loop has a higher potential to support *Daphnia* performance than tPOM. Although majority of terrestrial organic matter entering lakes is in the dissolved form, much of tDOM is recalcitrant and resistant to bacterial degradation thus supporting only low bacterial production (Tranvik, 1992; Räsänen et al., 2016). The labile, low molecular weight components of tDOM could support high bacterial growth rates and may offer a pathway of allochthonous organic matter to enter lake food webs (Berggren et al., 2010). However, bacteria may preferentially use autochthonous DOM exudates from phytoplankton cells, leading to lower utilization of tDOM (Kritzberg et al., 2005). Additionally, transfer of energy (and carbon) via the microbial pathway is significantly hindered by losses due to e.g. respiration with addition of several trophic steps, regardless of whether the substrate is autochthonous or allochthonous in origin (Berglund et al 2007). Even when assuming a relatively high growth efficiency of 20-30% on each trophic level (Straile 1997; Kritzberg et al., 2005), the tDOM-bacteria-heterotrophic flagellate-*Daphnia* food web would result in only <3% of carbon reaching *Daphnia*. Combining this with the high growth efficiency of *Daphnia* grazing on phytoplankton (up to ~40%, Brett et al., 2009) and the 10-fold higher biomass of phytoplankton than heterotrophic flagellates (Auer et al., 2004), the overall importance of tDOM in supporting zooplankton when phytoplankton is abundant, e.g. in spring and summer, is likely low. Nevertheless, there is potential for microbial loop supplementing zooplankton diets at times, e.g. during autumn and winter when phytoplankton production is low or when phytoplankton community is composed of low quality taxa, and our results indicate that heterotrophic flagellates may be key taxa in supplying zooplankton with essential biochemical components.

Conclusions

We studied how a microbial food web based upon terrestrial DOM supported *Daphnia* growth and reproduction. While the *Cryptomonas* diet was clearly best at supporting *Daphnia* growth and reproduction, the heterotrophic flagellate *Paraphysomonas*-microbial food web diet stemming from tDOM resulted in similar performance measures for *Daphnia* as those fed upon the green alga

Scenedesmus. The *Paraphysomonas* diet also led to higher survival, growth and reproduction of *Daphnia* than a diet of cyanobacteria, most likely due to essential PUFA and sterols that are synthesized by *Paraphysomonas*. Due to inefficiency of energy transfer across multiple trophic steps in the microbial food web, it is unlikely that tDOM would support a large fraction of zooplankton production, and certainly not during the spring or summer when better quality food abounds. However, heterotrophic flagellates may be important in providing zooplankton with essential biochemical components when phytoplankton quantity and/or quality is low, e.g. during autumn and winter, or cyanobacterial blooms, respectively.

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Table and Figure legends.

Table1. Origin, culture media, and cell lengths (mean ± SD) of the diet items.

Table 2. Sterol composition (%), total concentration (μg mg $DW^{-1,}$) and sterol index in diets (mean of 2-3 replicate analysis). nd = not detected.

Fig. 1. Survival of *Daphnia* in diet treatments. *Paraphysomonas* treatment lasted for 12 days, other treatments 14 days. *n* = 10 in the beginning.

Fig. 2. A) Somatic growth rate (*n* = 5) and B) length (*n* = 5-10) of *Daphnia* in diet treatments. *Paraphysomonas* treatment lasted for 12 days, other treatments 14 days. Treatments indicated with same letters belong to homogenous subsets (ANOVA, posthoc Tukey B).

Fig. 3. Mean number of neonates produced by *Daphnia* in the diet treatments. No reproduction too place before day 9. *Paraphysomonas* treatment lasted for 12 days, other treatments 14 days. Eggs and embryos in the brood pouch of *Paraphysomonas*-fed *Daphnia* are included in the graph as "potential neonates" for day 14 (dashed grey line). *n* = 5-10, and only *Daphnia* alive at the end of the experiment were included in calculating the mean.

Fig. 4. NMS ordination of percent fatty acid composition of *Daphnia* and different diets offered in the experiment. Filled markers = *Daphnia*, open markers = diets. Fatty acids of *Daphnia* were analyzed for days 7 and 14 (not separated in figure), and diets only once. *n* = 2-5 per treatment for *Daphnia* and *n* = 3 for diets. Fatty acids that correlate strongly (Pearson $r > 0.4$) with either axis are presented as vectors. Unknown double-bond locations of fatty acids marked with ωn. I/ai-branched = sum of iso- and anteiso-branched fatty acids.

Tables

Table 1.

Diet item	Code	Origin	Culture medium	Cell length (µm)
Cryptomonas marssonii	Crypto	CCAP[a] 979/70	WC (Guillard, 1975)	17.0 ± 3.2
Scenedesmus gracilis	Scene	University of Basel	L16 (Lindström, 1983)	15.9 ± 3.6
Microcystis sp. strain 130	Micro	University of Helsinki	Z8 (Staub, 1961)	5.6 ± 2.0
Paraphysomonas vestita	Para	SCCAP[b] K-1213	DY-V[c] (Andersen et al., 2005)	8.5 ± 2.3

[a] The Culture Collection of Algae and Protozoa, UK

[b] The Scandinavian Culture Collection of Algae and Protozoa, Denmark

[c] *Paraphysomonas* was cultured with lake bacteria growing on peat extract.

541 Table 2.

Sterol	*Micro*	*Scene*	*Crypto*	*Para*
Fucosterol	nd	5.1	nd	nd
Fungisterol	nd	23.5	nd	nd
Chondrillasterol	nd	58.6	nd	nd
Dihydrochondrillasterol	nd	3.4	nd	nd
Schottenol	nd	9.5	nd	nd
Brassicasterol	nd	nd	37.2	5.0
Campesterol	nd	nd	5.7	1.7
Stigmasterol	nd	nd	41.8	0.6
β-sitosterol	nd	nd	15.2	92.8
µg mg DW^{-1}	nd	1.6	4.2	1.9
Sterol index	nd	0.21	1.21	0.23

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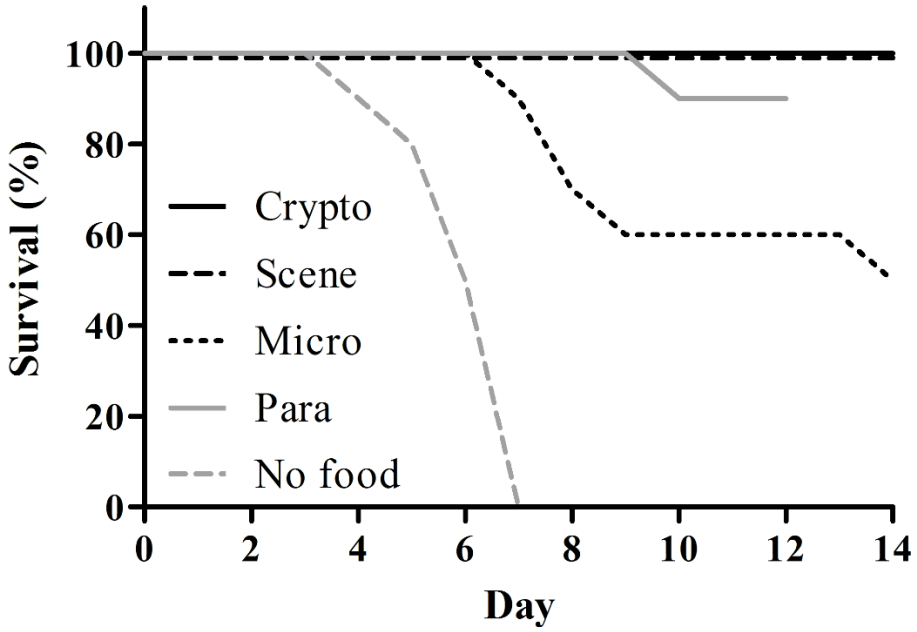

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545 Fig.1

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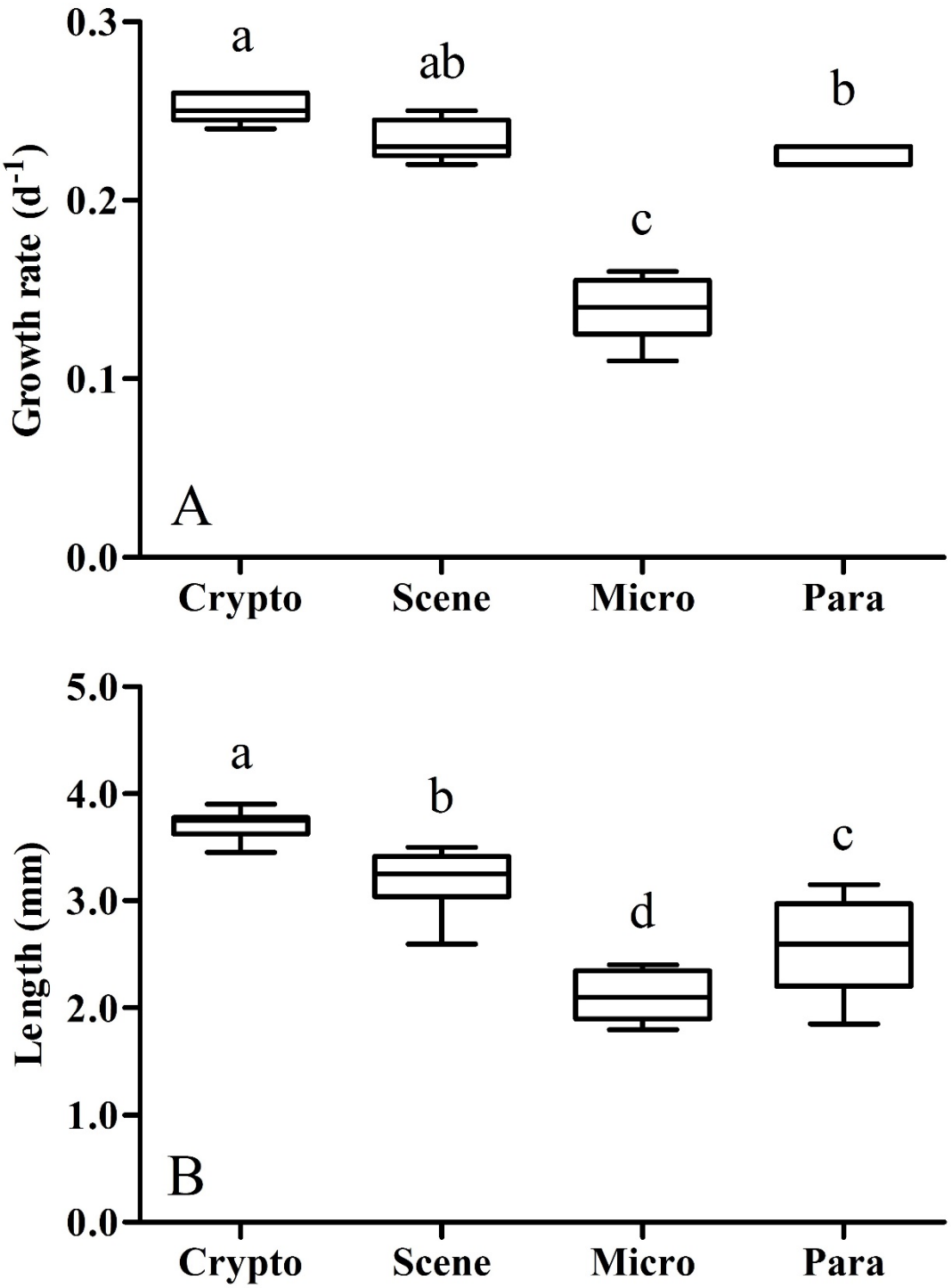
0.3
0.2
0.1
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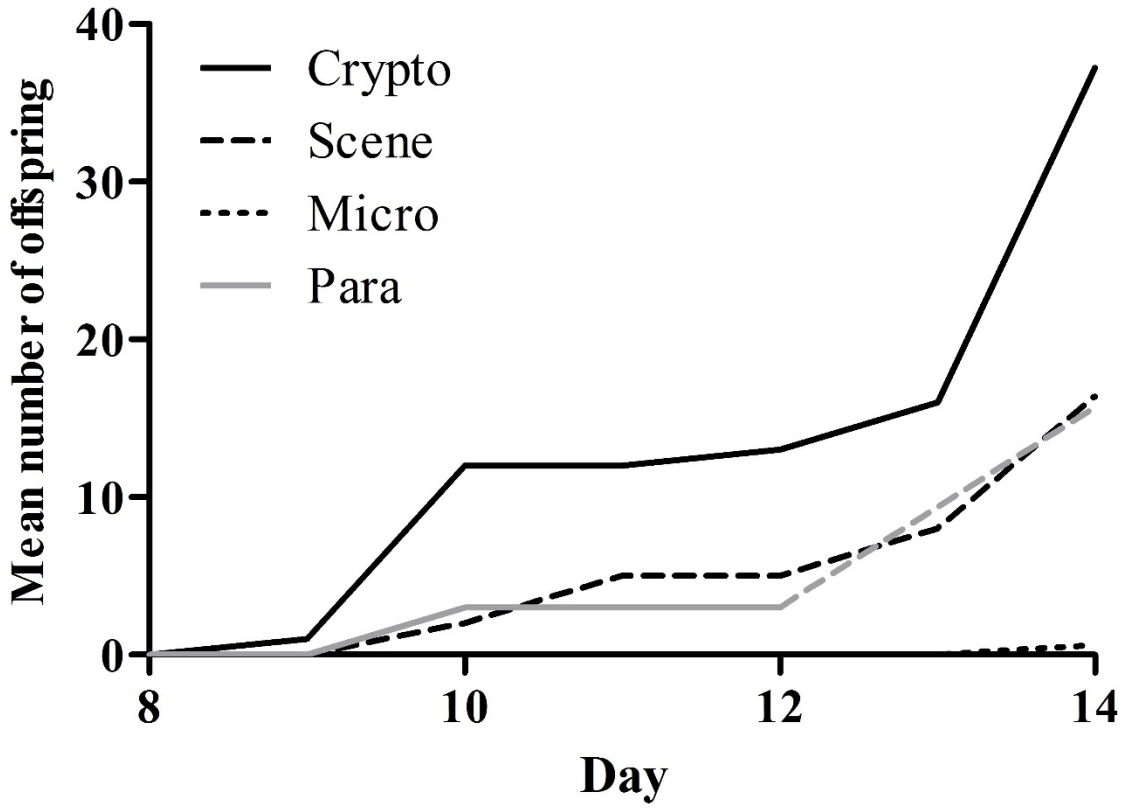
Growth rate (d-1)
a
ab
b
c
A
Crypto
Scene
Micro
Para
5.0
4.0
3.0
2.0
1.0
0.0
Length (mm)
a
b
c
d
B
Crypto
Scene
Micro
Para

Fig. 2.



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551 Fig. 3.

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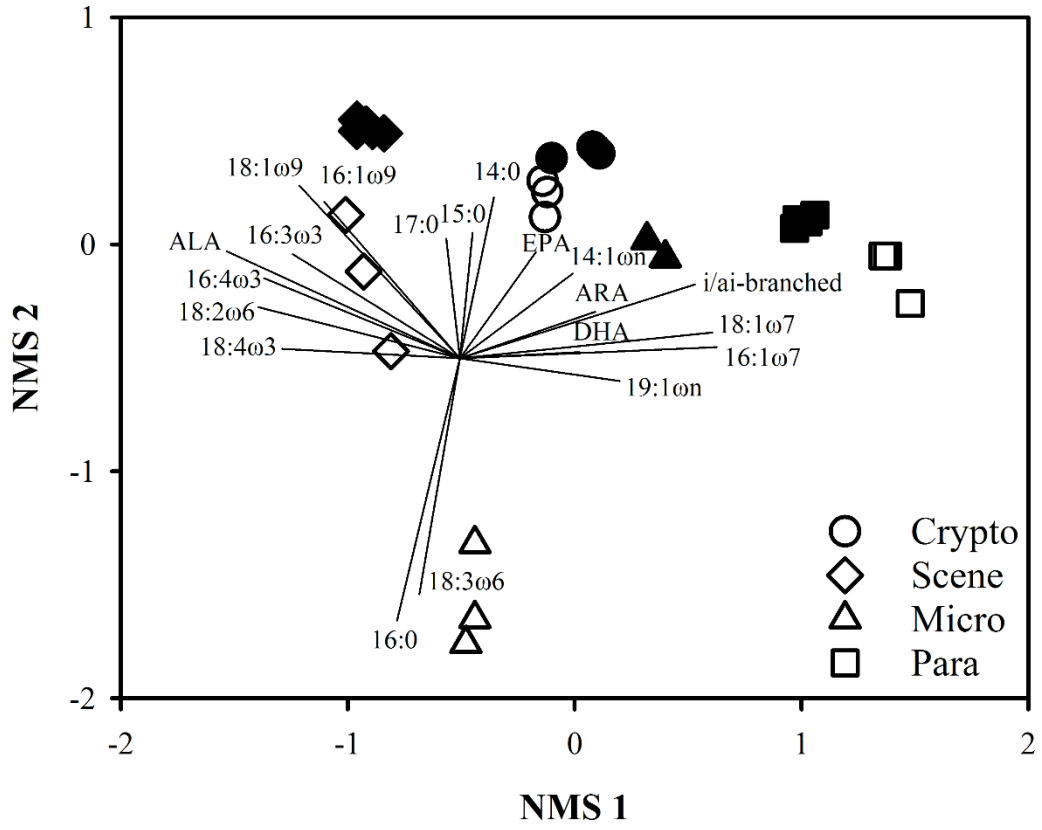
1
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2
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NMS 1
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17:0
15:0
EPA
14:1ωn
ALA
16:3ω3
16:4ω3
18:2ω6
18:4ω3
ARA
i/ai-branched
DHA
18:1ω7
16:1ω7
19:1ωn
18:3ω6
16:0
Crypto
Scene
Micro
Para

553

554 Fig. 4.